EPCAM ANTIBODY AND CAR-T CELLS

[0001] This application claims priority to U.S. Provisional Application No. 63/009,018, filed Apr. 13, 2020; the contents of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING, TABLE OR COMPUTER PROGRAM

[0002] The Sequence Listing is concurrently submitted herewith with the specification as an ASCII formatted text file via EFS-Web with a file name of Sequence Listing.txt with a creation date of Apr. 12, 2021 and a size of 11.5 kilobytes. The Sequence Listing filed via EFS-Web is part of the specification and is hereby incorporated in its entirety by reference herein.

FIELD OF THE INVENTION

[0003] The present invention relates to EpCAM antibody and EpCAM-CAR-T Cells, which are useful in the field of adoptive immunity gene therapy for tumors. The invention particularly relates to chimeric antigen receptors comprising EpCAM single-chain variable fragment having functionally sufficient but low affinities to EpCAM, which mitigate cytotoxicity to normal tissues.

BACKGROUND OF THE INVENTION

[0004] Immunotherapy is emerging as a highly promising approach for the treatment of cancer. Genetically modifying T cells with CARs is a common approach to design tumor-specific T cells. CAR (chimeric antigen receptor)-T cells targeting tumor-associated antigens can be infused into patients (adoptive cell transfer or ACT) representing an efficient immunotherapy approach. The advantage of CAR-T technology compared with chemotherapy or antibody is that reprogrammed engineered T cells can proliferate and persist in the patient and work like a living drug.

[0005] CAR molecules are composed of synthetic binding moieties, typically an antibody-derived single chain fragment variable (scFv) or any native antigen-sensing element, fused to intracellular signaling domains composed of the TCR zeta chain and costimulatory molecules such as CD28 and/or 4-1BB[1, 2]. The advantages of CAR mediated targeting include: 1) the provision of activation, proliferation, and survival signals in-cis via a single binding event, compared to the natural, non-integrated TCR and costimulatory signaling; 2) the ability to bypass the downregulation of MHC by tumor cells through MHC-independent antigen recognition; and 3) a reduced activation threshold as well as recognition of tumor cells with low antigen density enabled by the high affinity interaction between CAR and antigen [3, 4].

[0006] The ideal CAR target antigen would be a native, surface-exposed tumor neoantigen that is highly expressed in tumor tissues and is undetectable in healthy tissues. However, due to the implicit rarity of such antigens, many commonly targeted solid tumor antigens, are also expressed by non-tumor tissues, albeit at lower levels. CAR molecules with high affinity to such antigens can lead to collateral targeting of healthy tissues resulting in on-target, off-tumor toxicity, a major limiting factor to the progress of CAR T cell therapy to date.

[0007] Conventional CARs are constructed using a single-chain antibody format, and are selectively engineered to possess sub- to low nanomolar affinities for target antigens. However, increased CAR T cell sensitivity may be an advantage only when targeting true tumor antigens or those with the highest levels of restriction. Otherwise, increased sensitivity comes at the price of reduced selectivity with lysis of target-expressing cells in a manner largely insensitive to antigen density.

[0008] EpCAM (Epithelial Cell Adhesion Molecule) (CD326) antigen is a 39-40 kDa cell surface glycoprotein that is encoded by EpCAM gene. EpCAM plays a crucial role in cell adhesion, growth, proliferation, inflammation, cancer and metastasis. EpCAM is highly overexpressed in many types of tumors such as breast cancer, ovarian cancer, non-small cell lung cancer, pancreas cancer, stomach cancer, colon cancer and colorectal cancer. EpCAM is also expressed in many normal tissues but its expression in tumor tissues is significantly higher.

[0009] The use of T cells to fight cancer is dependent upon optimally activated T cells, whether they are endogenous or genetically engineered. Continuous exposure of T cells to antigen results in their exhaustion, a state characterized by the deterioration of cellular functions. Exhausted T cells display loss of effector functions, begin to express multiple inhibitory proteins and are defined by an altered transcriptional repertoire.

[0010] High affinity EpCAM CAR-T cells recognize epithelial cell adhesion molecule-expressing cells: both normal epithelial tissues with low levels of EpCAM, and carcinomas expressing it at considerably higher levels. The recognition of antigen both on normal, non-target cells as well as on cancer cells can lead to both unwanted toxicity and T cell exhaustion.

[0011] There exists a need for CARs with improved therapeutic index, i.e., CARs that can kill tumor while minimizing systemic toxicity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. **1**A shows the variable regions of the heavy chain (V_H, SEQ ID NO: 1) of UBS-54 huMab. FIG. **1**A also shows the CDR-H3 of UBS-54 (SEQ ID NO: 2) and CDR-H3 of four variants with alanine substitution in CDR-H3. D1A: SEQ ID NO: 3. F3A: SEQ ID NO: 4. L4A: SEQ ID NO: 5, Y6A: SEQ ID NO: 6.

[0013] FIG. **1**B shows the schematic demonstration of the EpCAM targeting CAR construct. The scFv component is the sequence from UBS-54 huMab or one of the alanine substitution variants (D1A, F3A, L4A, and Y6A). LTR=long terminal repeat; SS=signal sequence; scFv=single-chain variable fragment; TMCyto=transmembrane and cytosolic domain.

[0014] FIG. **1**C shows the recombinant EpCAM binding to MYC-tagged CARs expressed in HEK293T cells. X axis: binding of Alexa Fluor 647 labeled recombinant EpCAM. Y axis: binding of anti-MYC antibody.

[0015] FIG. **1**D shows effector to target (E:T) assays for measuring target killing by primary T cells transduced with different EpCAM targeting CARs. Each target was separately incubated with different CAR T cells or non-transduced T (NT) cells at 2.5:1 E:T ratio. Percent of viability was normalized to luminescence from target cell only.